Cooperative Agreement No. 19-205

Title. INFLUENCE OF MICROORGANISMS ON THE PHEROMONAL REGIME OF THE SOUTHERN PINE BEETLE

Progress Report for

July 1, 1977 to December 31, 1977

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1. Laboratory Bioassays of Yeast Metabolites

Once again, during this period, compounds identified from microorganisms associated with Dendroctonus frontalis have been bioassayed in the walking laboratory bioassay of Tom Payne of Texas A & M.

2-Phenylethanol

In previous bioassays it appeared that this compound could inhibit the response of walking female beetles. This possibility was discussed in the last progress report. The bioassays were therefore extended to cover a wider range of concentrations of 2-phenylethanol in order to assess the limits at which this effect might still be evident. If one assumes that this alcohol is produced in the phloem of attacked trees, must it be produced in large amounts in order to exhibit an effect? A lot of money and effort has been put into attempts to show that endo-brevicomin exhibits an inhibitory effect in the field. Bioassays were therefore done using a fixed concentration of triplicate standard and ever decreasing concentrations of added 2-phenylethanol. These results are presented in Table 1.

It is evident that 2-phenylethanol does inhibit the walking response of female beetles. The percent response is decreased at all levels tested, except the final concentration of 10^{-6} times the concentration of triplicate standard.

These results seem to be particularly important. To be able to inhibit the fairly good response of females to a concentration of 10^{-0} triplicate standard at concentrations 10^{-5} less than the triplicate standard indicates that this aromatic alcohol could well act as a behavioral chemical in the natural environment of the beetle. Additional finances will be required to do field tests to establish the validity of these results in a natural situation.

2-Phenylethyl acetate and Isoamylacetate

These esters have been bioassayed in the same manner as 2-phenylethanol. The results confirmed those obtained previously suggesting that the attraction of males is enhanced by the addition of these substances. Appreciable enhancement was brought about by a concentration of ester 100 times lower than that of the triplicate standard (see Tables 2 and 3).

6-Methyl-5-hepten-2-one and 6-Methyl-5-hepten-2-ol

The mycangial fungus, SJB-122, produces both of these substances (see enclosed reprint). The results of walking laboratory bioassays on these two compounds are presented in Table 4. The response of males to the triplicate standard is significantly decreased by the addition of a 1:1 mixture of the ketone and alcohol at a concentration up to 100 times less than that of the triplicate standard. The fact that this

reduction on attractancy is due to the ketone and not to the alcohol is established from the results on the two compounds separately (see Table 4).

At this stage, and with only these few bioassays to go by, one can only speculate that 6-methyl-5-hepten-2-one, if produced by SJB-122 in attacked trees, could have some effect on the behavior of male beetles attracted to these trees. This compound should also be field tested.

Acetoin and 2,3-Butanedione

Previous bioassays on these compounds had suggested that they may be effective inhibitors. However, the bioassays reported in Tables 5 and 6 do not support the previously obtained data.

2. Volatile Products of SJB-122

A reprint is enclosed that reports on the identification of the major volatile products present in a steam distillate of a liquid culture of this fungus. Work has continued on the attempted identification of the minor components that are detectable. One of the early eluting compounds appears to be valeraldehyde. This identification is based on the mass spectrum of this peak.

There are four more minor peaks eluting after the major ones on a temperature programmed gas chromatogram of the steam distillate extract. Two of these substances give mass spectra similar to nerol and geraniol but positive identifications have not yet been made. Such compounds would account for a part of the quite pleasant fruity odor that cultures of this fungus often have.

TABLE 1

Response of D. frontalis to yeast metabolites in a laboratory bioassay.

2-phenylethanol

Compound	Percent response[2] (No. beetles tested) Males	Females
Standard[1]	59.9 (90)	53.6 (140)
Standard + 10^{0} yeast	----	40.0 (80)
" + 10^{-1} "	----	20.0 (30) ↓
" + 10^{-2} "	----	40.0 (30)
" + 10^{-3} "	31.7 (60) ↓	42.8 (35)
" + 10^{-4} "	50.0 (50)	26.7 (30) ↓
" + 10^{-5} "	50.0 (30)	30.0 (30) ↓
" + 10^{-6} "	60.0 (30)	68.0 (50)
Pentane	14.3 (35)	9.1 (55)

1. Standard is a 1:1:12 mixture of frontalin, trans-verbenol and turpentine. Bioassays of males used a standard concentration of 10^{-3}; bioassays of females used a 10^{0} concentration of the standard.
2. Those values marked with an arrow indicate a significant reduction or enhancement of response (indicated by the direction of the arrow) based upon one-way ANOV.

TABLE 2

Response of D. frontalis to yeast metabolites in a laboratory bioassay.

2-phenylethylacetate

Compound	Percent response[2] (No. beetles tested) Males		Females	
Standard[1]	60.0	(30)	53.3	(120)
Standard + 10^{-2} yeast	----		53.3	(30)
" + 10^{-3} "	----		60.0	(30)
" + 10^{-4} "	73.3	(30)	63.3	(30)
" + 10^{-5} "	83.3	(30) ↑	55.0	(40)
" + 10^{-6} "	53.3	(30)	50.0	(50)
" + 10^{-7} "	----		66.7	(30)
Pentane	1.0	(30)	2.5	(40)

1. Standard is a 1:1:12 mixture of frontalin, trans-verbenol, and turpentine. Males were bioassayed at a 10^{-3} and females were bioassayed at 10^{0}.
2. Values marked with an arrow indicate a significant reduction or enhancement of response (indicated by direction of arrow) based upon one-way ANOV.

TABLE 3

Response of D. frontalis to yeast metabolites in a laboratory bioassay.

Iso-amylacetate

Compound	Percent response (No. beetles tested) Males	Females
Standard[1]	65.0 (60)	51.0 (100)
Standard + 10^{0} yeast	----	66.7 (30)
" + 10^{-1} "	----	63.3 (30)
" + 10^{-2} "	----	66.7 (30)
" + 10^{-3} "	56.7 (30)	50.0 (30)
" + 10^{-4} "	76.7 (30)	56.7 (30)
" + 10^{-5} "	80.0 (30)	43.3 (30)
" + 10^{-6} "	76.7 (30)	33.3 (30)
" + 10^{-7} "	66.7 (30)	30.0 (30)
Pentane	10.0 (20)	11.4 (35)

1. Standard is a 1:1:12 mixture of frontalin, trans-verbenol, and turpentine. Males were bioassayed at a 10^{-3} concentration and females were bioassayed at 10^{0}.

TABLE 4

Laboratory bioassays of 6-methyl-5-hepten-2-one and 6-methyl-5-hepten-2-ol.

Treatment		$\bar{X} \pm$ SE % response Male	95% confidence intervals
Standard (10^{-1})		60 ± 5	51 - 69
Methylheptenone + Methylheptenol	10^{-2}	23 ± 6	11 - 35
	10^{-3}	23 ± 10	4 - 42
	10^{-4}	43 ± 6	31 - 55
	10^{-5}	63 ± 6	51 - 75
	10^{-6}	60 ± 5	50 - 70
	10^{-7}	50 ± 7	37 - 63
Pentane		4 ± 3	0 - 10
Standard (10^{-1})		54 ± 5	46 - 63
Methylheptenone	10^{-2}	20 ± 7	6 - 34
	10^{-3}	17 ± 6	5 - 29
	10^{-4}	70 ± 7	57 - 83
	10^{-5}	73 ± 7	60 - 86
	10^{-6}	60 ± 5	50 - 70
	10^{-7}	60 ± 5	50 - 70
Pentane		0 ± 0	-------
Standard (10^{-1})		65 ± 8	50 - 80
Methylheptenol	10^{-2}	~~47 ± 7~~	~~33 - 61~~
	10^{-3}	~~60 ± 10~~	~~48 - 72~~
	10^{-4}	57 ± 8	41 - 72
	10^{-5}	90 ± 5	81 - 99
	10^{-6}	67 ± 8	50 - 83
	10^{-7}	53 ± 8	37 - 70
Pentane		10 ± 6	0 - 21

TABLE 5

Laboratory bioassays of Acetoin[1]

$\bar{X} \pm$ SE % responding beetles and 95% confidence intervals

Treatment	Male	$CI_{.95}$	Female	$CI_{.95}$
Standard[2]	54 ± 4	46 - 61	66 ± 5	56 - 74
Acetoin 10^{0}	67 ± 10	47 - 86	------	-------
10^{-1}	63 ± 10	45 - 82	------	-------
10^{-2}	70 ± 9	53 - 87	55 ± 4	47 - 62
10^{-3}	70 ± 5	61 - 79	69 ± 7	56 - 82
10^{-4}	67 ± 8	50 - 83	63 ± 11	42 - 84
10^{-5}	70 ± 11	48 ± 92	60 ± 10	40 - 80
10^{-6}	60 ± 5	50 - 70	-------	-------
Pentane	1 ± 1	0 - 4	13 ± 7	0 - 26

[1] Bioassays of males were conducted from August 30 - September 8; those for females were conducted from May 25 - 27.

[2] Standard was a 1:1:12 mixture of frontalin trans-verbenol and loblolly turpentine. Bioassays of the standard were run at 10^{-1} and 10^{0} concentration of the standard for male and female SPB respectively.

TABLE 6

Laboratory bioassays of 2,3-butanedione[1]

Treatment		$\bar{X} \pm$ SE % response and 95% confidence interval			
		Male	$CI_{.95}$	Female	$CI_{.95}$
Standard[2]		58 ± 3	51 - 64	45 ± 4	38 - 53
2,3-butanedione	10^{0}	47 ± 10	27 - 66	33 ± 8	17 - 50
	10^{-1}	63 ± 6	51 - 75	40 ± 7	26 - 54
	10^{-2}	53 ± 10	34 - 73	43 ± 11	22 - 64
	10^{-3}	58 ± 12	34 - 82	60 ± 9	43 - 77
	10^{-4}	65 ± 7	51 - 79	53 ± 4	45 - 61
	10^{-5}	72 ± 6	60 - 83	40 ± 5	30 - 50
	10^{-6}	68 ± 3	62 - 74	43 ± 13	17 - 69
Pentane		17 ± 11	0 - 38	15 ± 6	3 - 27

[1] Bioassays of males were conducted from June 20 to August 26; female were run from June 1 to June 8.

[2] Standard was a 1:1:12 mixture of frontalin, trans-verbenol and loblolly turpentine. Bioassays of standard were run at 10^{-1} and 10^{0} for male and females respectively.